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(54) Title: DIRECT OVIDUCT TRANSGENESIS

(57) **Abstract**

Methods for preparing transgenic avians which express exogenous protein substantially only in their oviducts are disclosed. Each of the methods comprises delivering nucleic acid expression cassettes directly to the oviducts of the avians. The exogenous protein expressed by the expression cassette is secreted into the lumen of the avian oviduct and secreted into the eggs of the transgenic avians. Methods for preparing eggs which contain exogenous protein, such as human interferon, and methods for the production of proteins are also disclosed. The methods for direct oviduct transgenesis may also be used to assess the suitability of expression cassettes or exogenous proteins for expression in the avian oviduct.

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INTERNATIONAL SEARCH REPORT

Interr ial Application No
PCT/US 99/19393

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/00 A01K67/027 C07K14/56 C12N15/86 C12N7/04
C12N7/01 C12N15/21 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01K C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MURAMATSU, T. ET AL.: "Gene gun-mediated in vivo analysis of tissue-specific repression of gene transcription driven by the chicken ovalbumin promoter" MOLECULAR AND CELLULAR BIOCHEMISTRY, vol. 185, no. 1-2, 6 August 1998 (1998-08-06), pages 27-32, XP002095226	1,4,12
Y	the whole document	1-18, 22-25

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
1 February 2000	19. 04. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	CHAMBONNET, F

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna' al Application No
PCT/US 99/19393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OCHIAI H, PARK HM, NAKAMURA A, SASAKI R, OKUMURA JI, MURAMATSU T: "Synthesis of human erythropoietin in vivo in the oviduct of laying hens by localized in vivo gene transfer using electroporation." POULT SCI., vol. 77, no. 2, February 1998 (1998-02), XP000863530 cited in the application	1,4,12, 14
Y	the whole document	1-18, 22-25

Y	WO 97 47739 A (MACARTHUR WILLIAM C ;UNIV MICHIGAN (US); GENEWORKS L L C (US)) 18 December 1997 (1997-12-18) cited in the application claims 18-22	2,5,6, 16-18, 22-25

P,Y	WO 99 19472 A (AVIGENICS ;UNIV GEORGIA (US)) 22 April 1999 (1999-04-22) claims 19-29,35-39	1-18, 22-25

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 19393

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheets

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-18, 22-25

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/19393

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-18 22-25

A method for preparing a transgenic avian which expresses an exogenous protein substantially only in its oviduct, or which lays eggs containing exogenous protein, comprising: delivering a nucleic acid expression cassette directly to the oviduct of an immature avian, wherein the nucleic acid expression cassette comprises (i) a promoter and (ii) a nucleic acid sequence which codes for an exogenous protein and which is operably linked to the promoter; said transgenic avian.

2. Claims: 19-20

Methods for testing the efficiency of or the ability of a promoter to facilitate expression of a transgene in an avian oviduct comprising : delivering a nucleic acid expression cassette directly to the oviduct of an immature avian, wherein the nucleic acid expression cassette comprises (i) a promoter and (ii) a nucleic acid sequence which codes for an exogenous protein and which is operably linked to the promoter; and assaying for the presence or amount of the exogenous protein in the lumen of the oviduct.

3. Claim : 21

A method for screening a preparation of viral particles for deleterious mutations comprising: delivering the viral particles from a single preparation to the oviduct of an immature avian, wherein the viral particles contain nucleic nucleic acid expression cassettes comprising (i) a promoter and (ii)a nucleic acid sequence which codes for an exogenous protein and which is operably linked to the promoter; and assaying for the presence or amount of the exogenous protein in the lumen of the oviduct

4. Claims: 26-27

An intact avian containing protein exogenous to the avian egg.

5. Claim : 28

An Avian Leukosis Virus pseudotyped with the G envelope glycoprotein of the Vesicular Stomatitis Virus

6. Claim : 29 30

An isolated polynucleotide comprising the sequence set in SEQ ID NO:1, the complement thereof, an at least 12

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

nucleotide-long fragment thereof, or a sequence that hybridizes thereto, wherein said polynucleotide is not a fragment of an interferon alpha-2b found in nature; an expression vector comprising said nucleotide and a promoter linked thereto.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ial Application No
PCT/US 99/19393

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9747739 A	18-12-1997	AU 3479997 A	07-01-1998
		CA 2257871 A	18-12-1997
		EP 0960194 A	01-12-1999
WO 9919472 A	22-04-1999	AU 1189999 A	03-05-1999